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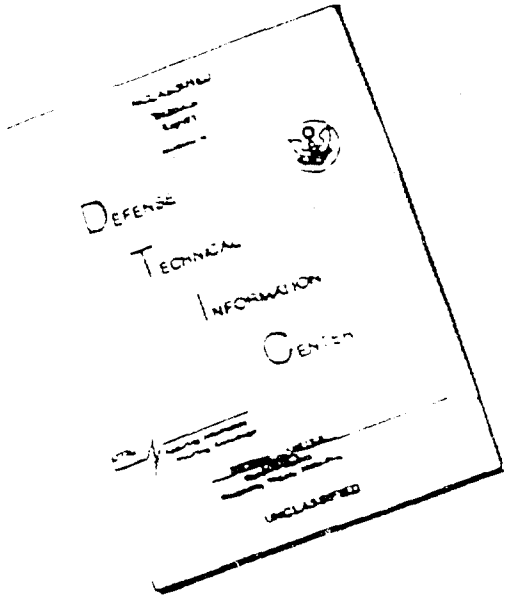

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CHARACTERISTICS OF THE POLYSACCHARIDE-CONTAINING FRACTION FROM PASTEURELLA PESTIS plus SUMMARY

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TRANSLATIONS FROM ZHURNAL MIKROBIOLOGII (Journal of Microbiology)

/Following are translations of two articles from the above-mentioned Soviet medical journal, No 6, 1962./

Characteristics of the Polysaccharide-containing Fraction from Pasteurella Pestis[1)]

by Ye. E. Bakhrakh, V. D. Yegorova, and L. P. Pavlova

State Scientific Research Institute of Microbiology and Epidemiology of the Southeastern USSR ("Mikrob")

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pages 126-130

The repeated attempts of obtaining from the plague microorganism a whole antigen, similar to the one isolated from a number of microorganisms by the method of Boivin and Mesrobean ended in failure (Girard, 1941; Korobkova, et al., 1944). Nevertheless, from the cells of the plague microorganism (the EV strain), following their hydrolysis with 0.1 N acetic acid, as per White method (1927), a specific polysaccharide-containing fraction was isolated (Bakhrakh, Korobkova, Shalayeva, 1958). The obtained preparation proved to be a hapten; it was not toxic to white mice and possessed no immunogenic properties, but in large dilutions it caused ring-precipitation with anti-plague sera, and it was recommended for titration of the latter (Dzhaparidze and Sidor-

1) Reported at the Scientific Conference of the Institute "Mikrob" on February 1960.

ova, 1956). The isolated fraction also induced a positive intradermal reaction in guinea pigs which had recovered from plague or had been immunized against this infection (Pavlova, 1958; Zaplatina and Konnova, 1956; Bakhrakh, Korobkova, Pavlova, Yegorova, 1960).

All this prompted us to undertake a study of the chemical composition of the above-mentioned fraction of the plague microorganism.

It is a known fact that the White method is the most widespread means of isolation of specific polysaccharides directly from the bacterial cell. The results, cited in the above-quoted works, of the chemical investigation of preparations isolated by means of this method from the vaccine strain of EV Past. pestis indicated the presence in them of a polysaccharide composed of glucose, glucosamine, and some unidentified sugar (an analogous composition of a polysaccharide obtained from Past. pestis via extraction with phenol was established by Davis, 1956). In the isolated preparations, together with the polysaccharide, a large percent of nitrogen was regularly detected, and by the method of chromatography on paper ten amino acids were identified.

On the basis of the data of previous works it was difficult to state with certainty whether the obtained preparation represents a stable protein-polysaccharide complex or whether it is a specific polysaccharide insufficiently purified of the protein admixture. The clarification of this question is of considerable importance, in connection with the possibility of utilizing the above-mentioned polysaccharide-containing fraction as an allergen for the elicitation of immunity to plague, and with the negative role which the ballast protein component plays in this fraction (Bakhrakh et al., 1960).

In the present work the polysaccharide-containing fraction was isolated from strain No 1 of Past. pestis grown on bouillon under conditions of active aeration at 28°C. The Past. pestis cells were separated from the bouillon via centrifugation, thrice irrigated with a physiological solution, precipitated on cold with acetone, and dried in a vacuum-exsiccator. The technique of isolating the polysaccharide fraction had been described and cited in the afore-mentioned works of Bakhrakh, Korobkova, Shalayeva (1958) and Bakhrakh, Korobkova, Pavlova, and Yegorova (1960).

The dry microbial cells were treated with 0.1 N acetic acid at 100° for one hour. The hydrolysate was steam-concentrated and fractionally precipitated with 1, 5, and 10 volumes of alcohol.

In the obtained preparations we determined the content of ash, nitrogen (by the method of isothermic distillation), and the total phosphorus (by the modified method of Fiske-Subarrou with ascorbic acid); the amount of reduced sugars was determined after hydrolysis of the preparation with sulfuric acid for five hours by the method of Hagedorn-Jensen, and glucosamine -- by the method of Elson-Morgan in

the modification of Gladyshev (1956). The presence of protein in the preparation was determined by a test with trichloracetic acid; the quantitative measure of the protein content was evaluated by the intensity of the biuret test (Stickland, 1951). Colorimetric tests were carried out in a FEK-M electrophotocolorimeter. The sugars and amino acids, which entered the composition of the investigated fraction (following its acid hydrolysis and corresponding processing), were determined by means of chromatography on paper with the use of the following systems of solvents: phenol -- water (80:20) and n-butyl alcohol -- water -- acetic acid (50:40:10). An aniline-phthalate reagent served as a developer in the determination of sugars, and 0.1% solution of ninhydrin in acetone -- for the determination of amino acids.

In Table 1 the results are cited of the chemical analysis of fractions obtained by means of precipitation with 1, 5, and 10 volumes of alcohol.

Table 1

Characteristics of fractions isolated from the No 1 strain of Past. pestis

Fraction	No of alcohol volumes upon precipitation	Nitrogen (in %)	Reducing sugars (in %)	Glucosamine (in %)	Phosphorus (in %)	Ashes (in %)	Titer of the precipitation reaction with antiplague serum
First	1	9.5	6.35	0.21	5.65	16	1:10,000
Second ...	5	7.65	30.3	1.1	0.7	3.5	1:100,000
Third	10	10.1	18.1	2.11	2.4	10.7	1:100,000

Analysis of the cited data shows that all obtained fractions contained a considerable amount of ash admixtures (the hydrolysates had not been subjected to dialysis prior to precipitation). Analysis of various forms of phosphorus in the first two fractions showed that it was virtually completely determined in the oxygen-soluble fraction and, hence, did not represent the nucleoprotein phosphorus.

In all fractions a considerable content of nitrogen was noted which would decrease from the 1st to the 2nd fraction, and then increase again in the 3rd fraction. The latter phenomenon can be explained by the fact that, upon high alcohol concentration, together with proteins there was a precipitation also of the disintegration products of the protein molecule -- peptides and amino acids. All

fractions, with the exception of the first, dissolved well in water; with their one percent solution a distinct reaction was obtained on protein with trichloracetic acid; the reaction was most intense with the first fraction and least intense with the second one.

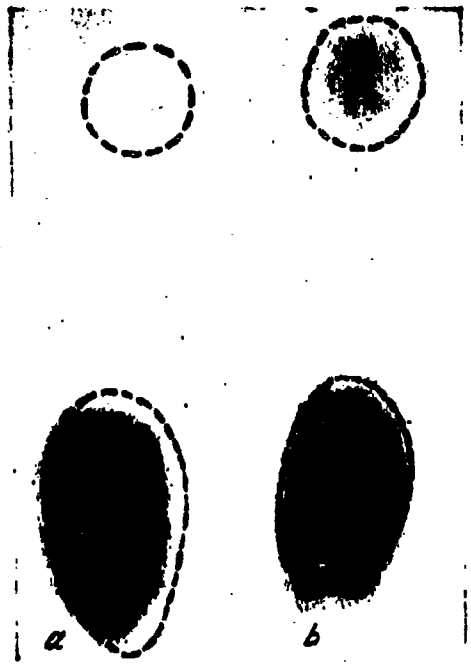

Fig. 1. Chromatogram of carbohydrate components of the polysaccharide-containing fraction

a - hydrolysate of the polysaccharide-containing fraction, b - witnesses /svideteli/; 1 - glucose, 2 - ribose

Thus, the purest polysaccharide fraction proved to be the one obtained via precipitation with five volumes of alcohol. It contained the smallest percentage of ashes and nitrogen and the highest ratio of reducing substances. By means of chromatography on paper, glucoses and traces of ribose were detected (Fig. 1).

We demonstrated in our previous work (Bakhrakh, Korobkova, Pavlova, and Yegorova, 1960) that, upon carrying out an allergic reaction on guinea pigs, the fraction obtained via precipitation with one volume of alcohol caused a postivie intradermal test not only in immunes,

but also in healthy animals, whereas fractions obtained by precipitation with five and ten volumes of alcohol caused an allergic reaction only in animals which had recovered from plague, or had been immunized against this infection. These data have been confirmed also in this work.

We did not subject the fraction obtained by precipitation with ten volumes of alcohol to a more detailed analysis, on account of the small amount of the obtained material, although the relatively high ratio of reduced sugars and glucosamine unquestionably rendered it interesting. We attempted, however, to free the protein component the fraction obtained by precipitation with five volumes of alcohol (second fraction).

It should be noted that the amount of protein in various preparations fluctuated within fairly wide limits. It depended on the conditions of hydrolysis and on the subsequent steps in the isolation of the polysaccharide-containing fraction. Thus, for example, a reduction of the microbial bodies concentration in the suspension, subjected to hydrolysis with acetic acid, led, together with an increased yield of the polysaccharide-containing fraction, to an increase of the nitrogen content in the preparations. A similar result was obtained upon the increase of hydrolysate concentration which had been achieved by means of boiling it prior to alcohol-precipitation of the polysaccharide-containing fraction.

The above tests have shown that a triple reprecipitation of the second fraction with alcohol led to the obtainment of a preparation, the 0.5 percent solution of which produced no reaction on protein with trichloracetic acid. In more concentrated solutions an addition of trichloracetic acid caused cloudiness. The nitrogen content of the preparations comprised 7.6%; that of protein, determined according to the intensity of the biuret test (Stickland, 1951), was 47.8%.

We attempted to precipitate the protein component with trichloracetic acid. For this purpose trichloracetic acid was added to the solution of the preparation, up to a final concentration of 4%. The precipitate was removed by means of centrifugation. The centrifugate was dialyzed, concentrated by evaporation, precipitated with five volumes of alcohol, and dried. No reaction with one percent solution of the preparation could be obtained with trichloracetic acid; however, its nitrogen content did not decrease appreciably (7.3%).

A similar result was obtained following repeated (up to eight times) purification of the polysaccharide-containing fraction with chloroform as per Sevag (1938) and a hydrolysis for a period of 12 hours with 0.1 N acetic acid. The latter method is employed, as is known, to separate glucidolipoid-protein complexes, isolated from the microorganisms by various methods. Our preparations obtained after

purification gave no positive reaction with trichloracetic acid, but they contained as previously a large amount of nitrogen (7.4--7.5%). Protein, determined as per Stickland, comprised 46-48%.

As to their serological activity and the property of inducing an allergic reaction in immune animals, the purified preparations did not differ from the initial ones. The trypsin-processing of the polysaccharide-containing fraction for 48 hours with subsequent dialysis and precipitation of the preparation with alcohol made it possible to reduce its protein component to 16.8%. However, a marked decrease of the allergenic properties of the preparation was simultaneously observed (Table 2).

By means of chromatography on paper cystine, lysine, aspartic acid, treonine, alanine, proline, thyrosine, valine, glycocoll, glutamic acid, leucine, glucosamine, glucose, and ribose were identified in the preparations isolated and purified of protein admixtures (Fig. 2).

Table 2

Allergenic properties of a polysaccharide-containing fraction of various purifications

Purification of the preparation	Protein (in %)	Result of the intradermal test in guinea pigs					
		immune			control		
		total	positive	negative	total	positive	negative
Triple reprecipitation with alcohol	47.8	10	10	--	5	--	5
Processing with trypsin	16.8	10	5	5	5	--	5

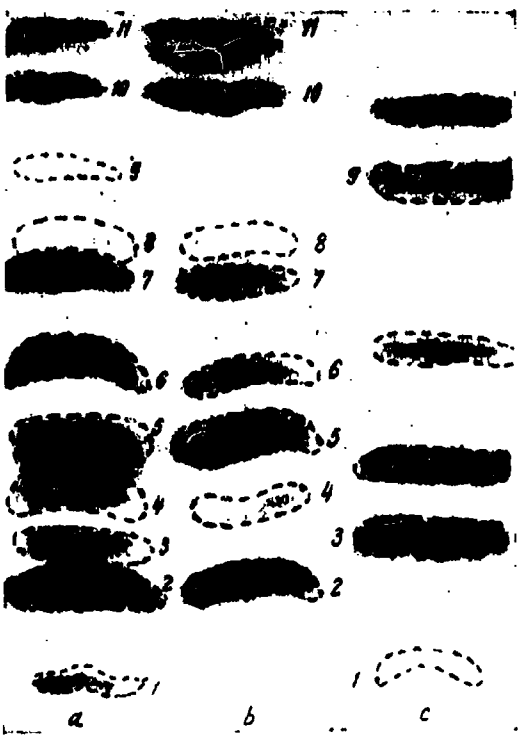

Fig. 2. Chromatogram of the amino acid composition of the polysaccharide-containing fraction

a - hydrolysate of the polysaccharide-containing fraction, b and c - witnesses /svideteli/; 1 - cystine, 2 - lysine, 3 - aspartic acid, 4 - glycocoll, 5 - glutamic acid, 6 - treonine, 7 - alanine, 8 - proline, 9 - thyrosine, 10 - valine, 11 - leucine

Conclusions

1. The preparation isolated by the White method from Past. pestis does not represent a pure polysaccharide. Non-purified fractions contained a considerable amount of protein admixtures, from which it could be separated via repeated, fractionated precipitation with alcohol, processing with chloroform as per Sevag, prolonged hydrolysis with 0.1 N acetic acid, or protein precipitation with trichloracetic acid.

2. The preparation purified of protein admixtures represents a stable polysaccharide-polypeptide complex possessing serological activity and the property of inducing allergic reaction in guinea pigs which had recovered from plague or had been immunized against this infection.

3. Removal of the polypeptide portion of the complex by means of trypsin-processing led to a marked reduction of the allergic effect of the preparation.

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Characteristics of the Polysaccharide-containing Fraction from Pasteurella Pestis[1)]

By Ye. E. Bakhrakh, V. D. Yegorova, and L. P. Pavlova

State Scientific Research Institute of Microbiology and Epidemiology of the Southeastern USSR ("Mikrob")

(Received by editors 4 July 1960)

The authors investigated the chemical composition of the specific polysaccharide-containing fraction of Past. pestis. The White method is the most prevalent means of isolating specific polysaccharides from a bacterial cell. The works of previous investigators of chemical compositions of preparations obtained by this method from the vaccine of EV strain of Past. pestis demonstrated the presence in them of a polysaccharide consisting of glucose, glucosamine, and some non-identified sugar (an analogous composition of a polysaccharide, obtained from Past. pestis via extraction with phenol, was established by Davis (1956). Together with a polysaccharide, the isolated preparations revealed a large percentage of nitrogen and, by means of chromatography on paper, 10 amino acids have been identified.

However, on the basis of the data of previous works it was difficult to ascertain reliably whether the obtained preparation represents a stable protein-polysaccharide complex or whether it is a specific polysaccharide insufficiently purified of the admixture of protein. The elucidation of this problem is of considerable importance in connection with the possibility of utilizing the afore-mentioned polysaccharide-containing fraction as an allergen for the elicitation of immunity in plague, as well as in regard to the negative role which the ballast protein-components play in this fraction.

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1) Reported at the Scientific Conference of the "Mikrob" Institute February 1960.

Conclusions

1. The preparation isolated by the White method from Past. pestis does not represent a pure polysaccharide. Non-purified fractions contained a considerable amount of protein admixtures, from which it could be separated via repeated, fractionated precipitation with alcohol, processing with chloroform as per Sevag, prolonged hydrolysis with 0.1 N acetic acid, or protein precipitation with trichloracetic acid.

2. The preparation purified of protein admixtures represents a stable polysaccharide-polypeptide complex possessing serological activity and the property of inducing a positive allergic reaction in guinea pigs which had recovered from plague or had been immunized against this infection.

3. Removal of the polypeptide portion of the complex by means of trypsin-processing led to a marked reduction of the allergic effect of the preparation.

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